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Original Article

Restless legs syndrome and central nervous system gamma-aminobutyric acid: preliminary associations with periodic limb movements in sleep and restless leg syndrome symptom severity

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ABSTRACT

Background: Previous research has demonstrated abnormalities in glutamate and *N*-acetyl aspartate (NAA) in the thalamus in individuals with restless legs syndrome (RLS) compared with healthy matched controls. However, levels of these transmitters in other RLS-related brain areas and levels of the most common inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), have not been assessed.
Methods: This study examined GABA, glutamate, and NAA levels in the dorsal anterior cingulate cortex (ACC), thalamus and cerebellum with the use of proton magnetic resonance spectroscopy (^{1}H-MRS) at 4 tesla (4 T) and Megapress difference-editing in 18 subjects with RLS and a matched control group without RLS. Actigraphy was performed on the nights before scans to assess periodic limb movements of sleep (PLMS).
Results: Levels of GABA, glutamate, and NAA were no different between RLS and control subjects in any of the three voxels of interest. However, GABA levels were positively correlated with both PLM indices and RLS severity in the thalamus and negatively with both of these measures in the cerebellum in RLS subjects. In addition, NAA levels were higher in the ACC in RLS than in controls.
Conclusion: Our preliminary data suggest that known cerebellar–thalamic interactions may modulate the intensity of RLS sensory and motor symptoms. In addition, anterior cingulate cortex may be associated with the affective components of the painful symptoms in this disorder.

1. Introduction

Restless legs syndrome (RLS) is characterized by an irresistible urge to move the legs, which is often associated with paresthesias. Central nervous systems (CNS) subserving sensory modulation, motor activity and pain appear to be altered in RLS [1]. In particular, leg discomfort and leg movements while awake in RLS are correlated with functional magnetic resonance imaging (fMRI)-related activation in the thalamus and cerebellum [2,3]. Similarly, enhanced dopaminergic binding [4] and a correlation of opioid binding with RLS disease severity [5] are seen in the thalamus in positron emission tomography (PET) studies in RLS. Ascending pain pathways project from the thalamus to the anterior cingulate cortex (ACC), which is involved in affective and cognitive aspects of pain [6]. ACC activity is abnormal in RLS by both fMRI [7,8] and PET [4,5].

Thalamic glutamate is 50% higher [9] and the neuronal marker *N*-acetyl aspartate (NAA) is reduced in the thalamus [10] in RLS using proton magnetic resonance spectroscopy (^{1}H-MRS). To date, no study has reported levels of gamma-aminobutyric acid (GABA), the major CNS inhibitory neurotransmitter, in patients with RLS.

The primary aim of this study is to quantify GABA, glutamate, and NAA levels in the ACC, thalamus and cerebellum in patients with RLS and matched controls. Levels of these transmitters/metabolites will be correlated to RLS severity and to objective measures of sleep and leg movement activity.

2. Methods

2.1. Participants

Adult (aged >18 years) subjects were recruited from the greater Boston, MA, area from May 2010 to April 2012. RLS subjects met diagnostic criteria from the International RLS Study Group (IRLSSG) [11], had a history of RLS symptoms at least 15 nights in the previous month, or, if treated, this frequency of symptoms before treatment was started, and had a history of significant sleep disturbance

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due to RLS as indicated by a score of at least 2 on question 5 on the RLS severity scale. Age- and sex-matched healthy control subjects without sleep complaints or a family history of RLS were also recruited. For RLS subjects, RLS-related medications were discontinued 48 h prior to PSG and benzodiazepines were discontinued a minimum of one week prior to PSG. Control subjects were not permitted to use CNS-active agents for two weeks prior to enrollment and for the duration of the study.

All subjects were evaluated with an unstructured clinical interview for history of sleep, psychiatric, and medical disorders. Baseline laboratories included urine toxicology and pregnancy testing (for female subjects). Exclusion criteria for all subjects included clinical evidence of any moderate-to-severe sleep disorder other than RLS (e.g. obstructive sleep apnea, insomnia, etc.); apnea–hypopnea index (AHI) >15 for all subjects; current or past (within the preceding year) diagnosis of alcohol or drug dependence/abuse; history of significant medical or neurologic illness including significant head trauma or loss of consciousness >30 min; body mass index >35 kg/m^2; consumption of >10 cigarettes/day, >2 caffeinated beverages/day, or >2 standard alcoholic drinks/day for a period >1 month within the preceding year; recent history of shift-work; contraindicated condition for MR scanning; and women who were pregnant, lactating, or planning to become pregnant during the study.

The study was approved by the Institutional Review Board of Partners Healthcare, the parent organization of Brigham and Women's Hospital and McLean Hospital, and carried out in accordance with the Declaration of Helsinki. All subjects received compensation for their participation in this study.

2.2. Actigraphy and polysomnography (PSG)

An actigraph (PAM-RL, Respironics, Pittsburgh, PA, USA) recorded limb movements in both legs from the subjects' bedtime until final wake time for four nights before, and the night of, the PSG. PSG channels included electroencephalograph (EEG), electrooculogram (EOG), submental electromyography (EMG), EMG of both anterior tibialis muscles (separate channels for each leg), oral/nasal airflow, nasal pressure, pulse oximetry, and respiratory effort. The PSG was analyzed for traditional sleep staging and PLM indices by the same experienced technologist.

2.3. Questionnaires and diary

All subjects were administered the Pittsburgh Sleep Quality Index (PSQI) [12] and the Beck Depression Inventory (BDI) [13] at the baseline visit. All subjects filled out diaries for two days prior to MRS scans, estimating sleep onset, time awake after sleep onset and total sleep time. RLS symptom severity on these two nights prior to MRS scans was assessed on this diary with a scale of 0 (no RLS symptoms) to 4 (very severe RLS symptoms). An IRLS severity scale [11], modified by adding the preface "since you stopped taking your RLS medication" for all questions, was filled out by RLS subjects on the morning of the MRS scan.

2.4. Magnetic resonance imaging

MRI was performed at 13:00–14:00 on the day following the PSG. After acquisition of spectral data from each of the three voxels during the MRS at 4 T, subjects were asked by study staff to verbally rate the level of RLS discomfort in their legs during the previous 20 min on a scale from 1 (no discomfort) to 10 (extreme discomfort).

Imaging and spectroscopy were performed on a whole-body 4 T magnetic resonance scanner (Varian/UNITYInova, Palo Alto, CA, USA) at McLean Hospital in Belmont, MA, USA. Data collection utilized a birdcage-design, radio-frequency (RF) head coil operating at 170.3 MHz for proton (XLR Imaging, London, Canada). Scout images confirmed optimal positioning, and unsuppressed water signal was shimmed to a global water line-width of ≤25 Hz. Subsequently, high-contrast T1-weighted anatomical images were taken in the sagittal and axial planes [echo time/repetition time, 6.2 s/11.4 ms; field-of-view, 24 × 24 × 8 cm (sagittal) and 22 × 22 × 16 cm (axial); read-out duration, 4 ms; receive band-width, ± 32 kHz; in-plane matrix size, 128 × 256 × 16 (sagittal) and 256 × 256 × 64 (axial); in-plane resolution, 0.94 × 1.9 mm (sagittal) and 0.94 × 0.94 mm (axial); read-out points, 512; slice thickness, 2.5 mm, flip-angle, 11°] for voxel positioning and image-based voxel tissue segmentation analysis.

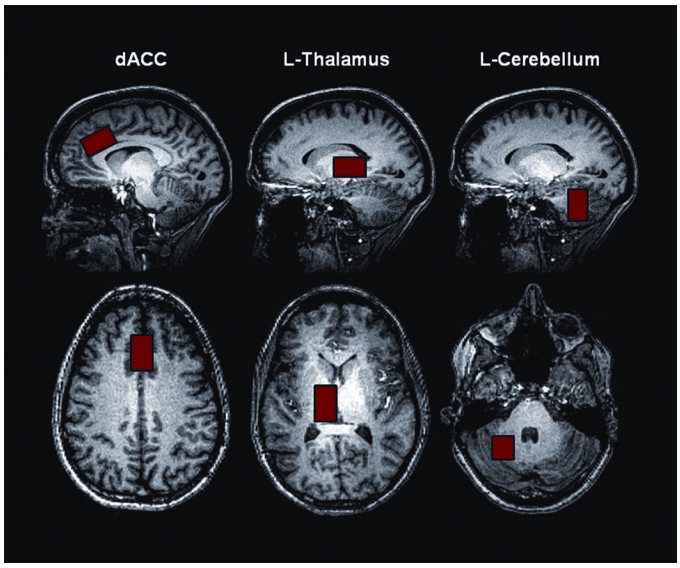

Fig. 1. Anatomical placement for dorsal anterior cingulate cortex (dACC), left thalamic (L-Thalamic) and left cerebellar (sub-vermis) (L-Cerebellum) proton magnetic spectroscopy voxels.

2.5. Proton MRS

The axial and sagittal high-resolution, T1-weighted anatomical images were used as a guide to systematically place single voxels in the left thalamic lobe (2 × 3 × 2 cm), bilateral dorsal ACC (3 × 2 × 2 cm), and left cerebellum (3 × 2 × 2 cm) (Fig. 1). Proton spectroscopy employed a GABA-optimized MEGA-PRESS sequence [14] for optimal measures of GABA using the difference-editing technique, as well as secondary measures of glutamate, NAA and total creatine (Cr) in the 68 ms sub-spectrum. Manual shimming of the magnetic field within each prescribed voxel achieved water line-widths ranging from 7 to 12 Hz. Following the automated optimization of water suppression power and tip angles, the transmitter frequency was set to the creatine resonance of 3.00 ppm to minimize chemical-shift displacement artifact for each spectral acquisition. The MEGA-PRESS sequence used the following acquisition parameters: TR, 2 s; TE, 68 ms; spectral band-width, 2 kHz; read-out duration, 512 ms; NEX, 384; and total scan duration, 13 min per voxel.

2.6. Proton MRS processing

All spectroscopic data processing and analyses were undertaken on a Linux workstation using reconstruction code written on-site (C-code) and commercial fitting software. In order to quantify difference-edited GABA with MEGA-PRESS data, the difference-edited spectra were fitted with LCModel [15,16] using basis sets acquired from phantoms at 4 T. All phase- and frequency-corrected 'on' and 'off' 68 ms sub-spectra were then averaged separately to

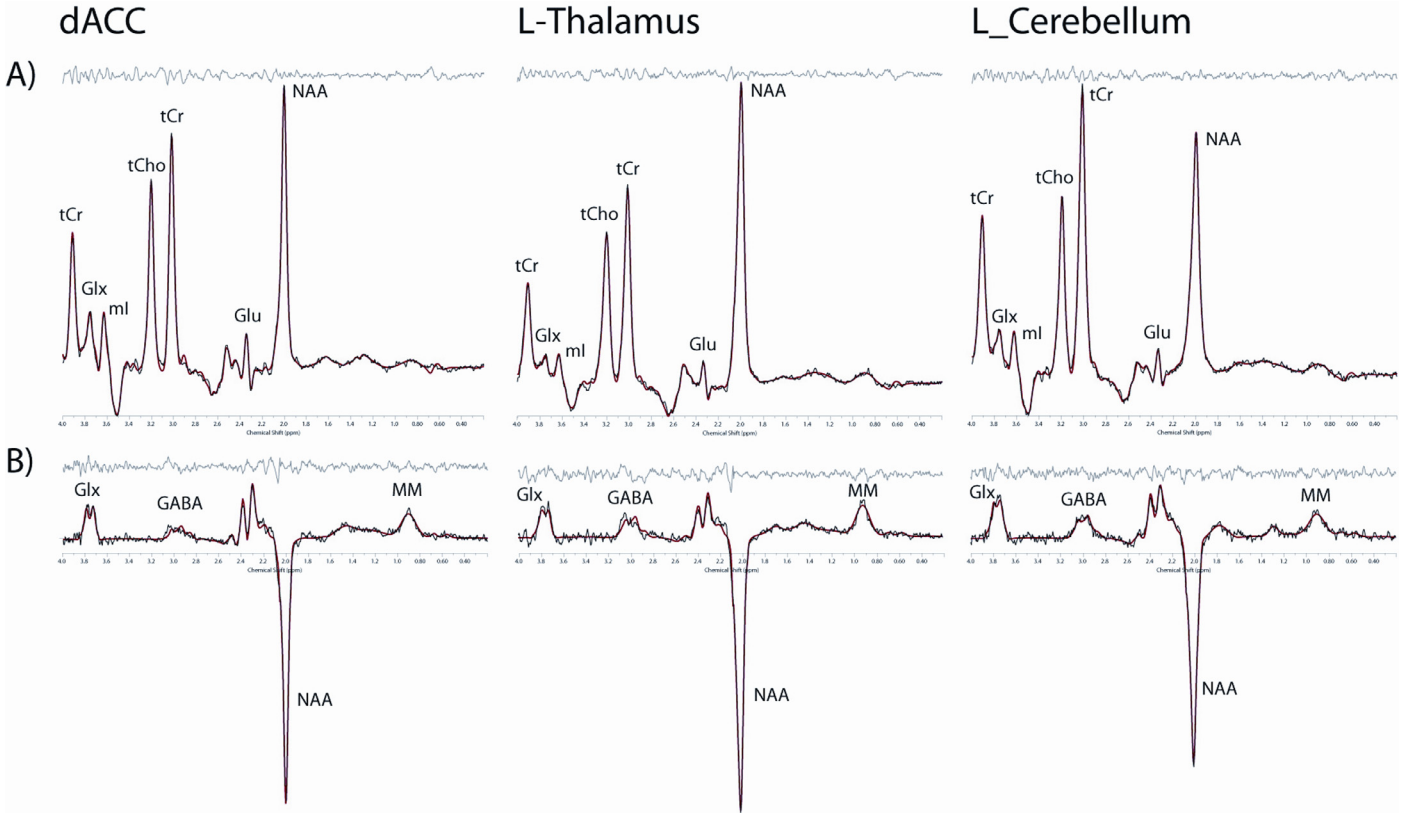

Fig. 2. MEGA-PRESS spectra from the dorsal anterior cingulate cortex (dACC), left thalamus and left cerebellum. Sixty-eight millisecond sub-spectra (A) and gamma-aminobutyric acid (GABA) difference-edited spectra (B) are shown with LCModel fit and residual for each region. tCr, total creatine; tCho, total choline; mI, myo-inositol; Glx, glutamate + glutamine; Glu, glutamate; NAA, *N*-acetyl aspartate MM, macromolecules.

produce a single 68 ms 'on' and 'off' spectrum, which were then subsequently subtracted to produce the final, optimized, difference-edited GABA spectrum. The appropriate phantom-based LCModel templates were used to fit the 68 ms 'off' spectrum for the measurement of creatine, glutamate and other metabolites (Fig. 2). The difference-edited GABA resonance area at 3.00 ppm, as well as the 68 ms 'off' spectrum metabolite areas, were normalized to the LCModel-fitted 68 ms 'off' spectrum creatine resonance area and left as simple ratios.

2.7. Image segmentation

To ascertain gray and white matter contribution to each voxel, the axial T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid compartments using FSL version 4.1 (FMRIB Software Library; Analysis Group, FMRIB; Oxford, UK) in combination with an in-house automated voxel co-registration and partial-volume analysis program.

2.8. Statistics

The primary statistical analysis was anaylsis of variance (ANOVA) with group and brain region as independent factors. Additionally, uncorrected post hoc *t*-tests were performed to examine differences between groups. The primary hypotheses were abnormal GABA/creatine, NAA/creatine and glutamate/creatine levels in the ACC, thalamus and cerebellum. Based on our previous GABA/Cr data obtained from the ACC in primary insomnia [17], we estimated 80% power to detect a difference between groups at $\alpha = 0.05$, two-sided with $n = 15$ in each group. Cramer–Rao % was used as an estimate of the signal:noise ratio. Voxels were considered outliers if Cramer–Rao % fell outside two standard deviations from the mean for all subjects. GABA/Cr data for individual subjects were excluded if LCModel was unable to fit GABA resonance.

Voxel tissue composition, demographics, psychometric scores, and diary/actigraphic data between groups were compared using unpaired *t*-tests (two-sided). Secondary analyses correlated GABA levels with the IRLSSG severity scale for the five days before scanning, RLS discomfort scores during the MRI, PSG-related sleep measures, sleep and RLS diaries for the two days before MRS scans, and PSG and actigraphy-related leg movement measures. Statistical analysis was performed using SPSS, Version 21.

3. Results

The RLS group ($n = 18$, all right-handed) was comprised of 10 women, with a mean age of 44.4 ± 15.0 (range, 18–64) years; the age- and sex-matched control group (one left-handed) had a mean age of 43.5 ± 14.3 (range, 19–64) years (Table 1). RLS subjects had duration of symptoms of 18.2 ± 16.1 years, with 14/18 reporting duration of symptoms ≥5 years (mean, 23.2 ± 15.8 years). Ten RLS subjects were taking medications for RLS prior to wash-out: eight on dopaminergic agonists, one on gabapentin and one taking both of these medications. The IRLS severity scale corroborated that they had moderate-to-severe RLS symptoms (Table 1). RLS subjects had significantly worse self-reported sleep on questionnaires and diaries. Polysomnography demonstrated numerically, though not statistically, worse sleep indices, though significant differences between groups were observed in PLM indices (Table 1). BDI scores were higher in RLS (mean, 7.7 ± 6) versus controls (mean, 2.1 ± 0.8) ($P = 0.001$), though still far below standard cut-offs for a clinical mood disorder and consistent with a typical pattern in insomnia

Table 1
Demographic and sleep–wake variables in restless leg syndrome (RLS) patients and controls.

Variables	RLS [mean (SD)]	Controls [mean (SD)]	*P*-value (two tailed)
Demographics			
Age (years)	44.4 (15)	43.5 (14.3)	NS
Sex (F:M)	10:08	10:07	
BMI (kg/m^2)	25.4 (3.5)	24.2 (2.8)	NS
Rating scales			
IRLS	23.3 (8.6)		
PSQI sleep latency	25.1 (20.8)	9.9 (7.1)	<0.05
PSQI total sleep time	6.2 (1.0)	7.8 (0.6)	<0.001
PSQI no. of awakenings	2.3 (1.0)	0.9 (1.0)	<0.001
PSQI sleep quality	1.3 (0.7)	0.2 (0.4)	<0.001
BDI	7.7 (6.0)	2.1 (0.8)	<0.001
Polysomnography			
SOL (min)	21.9 (23.5)	11.4 (6.1)	NS
WASO (min)	94.9 (70.6)	78.8 (50.2)	NS
PLMI	50.3 (47.4)	14.8 (25.4)	<0.05
PLMAI	20.3 (32.3)	1.7 (3.0)	<0.05
SE (%)	75.4 (14.0)	80.5 (11.7)	NS
Actigraphy			
PLMI final night	43.2 (50.4)	12.4 (14.6)	<0.05
PLMI average	45.1 (48.8)	10.8 (11.4)	<0.05
Diary (two-day average)			
SOL	80.1 (97.7)	18.1 (16.2)	<0.05
WASO	62.2 (60.8)	17.0 (11.4)	<0.05
TST	318.1 (118.3)	452.5 (42.3)	<0.001
RLS severity	2.3 (1.0)	0	<0.001

SD, standard deviation; BMI, body mass index; IRLS, International Restless Legs Syndrome questionnaire; PSQI, Pittsburgh Sleep Quality Index; BDI, Beck Depression Inventory; SOL, sleep onset latency; WASO, wake after sleep onset; PLMI, no. of periodic limb movements per hour in bed; PLMAI, no. of periodic limb movements with arousal per hour in bed; SE, sleep efficiency; TST, total sleep time.

(e.g. insomnia, irritability, fatigue). Baseline actigraphic data for one male control subject was not available due to device failure. Subject error resulted in partial actigraphic data for three RLS (two male) subjects and one male control subject.

One female RLS subject did not complete her scan and had no cerebellar data. Three subjects were re-scanned due to movement artifact or technical failure. Voxels excluded from GABA analysis due to Cramer–Rao outliers were: thalamus in three (RLS, one female; control, two male), cerebellum in five (RLS, one male; four control, two male), and ACC in two (RLS, two male). Voxels excluded from glutamate analysis due to Cramer–Rao outliers were: cerebellum in two (RLS, one male; control, one male) and ACC in two (RLS, one male; control, one male). Voxels excluded from NAA analysis due to Cramer–Rao outliers were: thalamus in three (RLS, one male, control, two female), cerebellum in one (control, one male), and ACC in three (RLS, one male; two control, one male). No subjects were excluded for structural MRI abnormalities.

3.1. Primary hypotheses

There were no differences in GABA/Cr or glutamate/Cr levels between RLS subjects and controls in the cerebellum, thalamus or ACC (Table 2). Post-hoc analysis excluding RLS subjects who were washed out of medications or whose RLS was <5 years' duration did not substantively alter the findings. Due to loss of sex-matching in the cerebellum and ACC from censored voxels, two-way ANOVA was performed to investigate the effects of sex on GABA and glutamate levels. There was no significant effect of diagnosis, sex or diagnosis × sex interaction in either region.

Analysis of NAA levels revealed significantly increased values in the ACC (RLS, 1.08 ± 0.09; controls, 0.098 ± 0.12; $P < 0.01$) though not in the cerebellum or thalamus (Table 2). There were no significant differences between RLS and controls for creatine, % gray matter, or % white matter in any region of interest.

Table 2
Four-tesla magnetic resonance spectroscopy metabolites in restless leg syndrome (RLS) patients and controls.

Region	Metabolite	RLS [mean (SD)]	Controls [mean (SD)]	*P*-value (two tailed)
ACC	GABA	0.19 (0.09) ($n = 16$)	0.19 (0.07) ($n = 17$)	NS
	Glutamate	0.95 (0.14) ($n = 17$)	0.97 (0.16) ($n = 16$)	NS
	NAA	1.08 (0.09) ($n = 17$)	0.98 (0.12) ($n = 15$)	<0.01
Thalamus	GABA	0.30 (0.12) ($n = 17$)	0.28 (0.08) ($n = 15$)	NS
	Glutamate	1.02 (0.28) ($n = 18$)	1.10 (0.33) ($n = 17$)	NS
	NAA	1.46 (0.17) ($n = 17$)	1.45 (0.16) ($n = 15$)	NS
Cerebellum	GABA	0.19 (0.07) ($n = 16$)	0.21 (0.09) ($n = 13$)	NS
	Glutamate	0.68 (0.26) ($n = 16$)	0.58 (0.13) ($n = 16$)	NS
	NAA	0.80 (0.20) ($n = 17$)	0.72 (0.09) ($n = 16$)	NS

ACC, anterior cingulate cortex; GABA, gamma-aminobutyric acid; NAA, *N*-acetyl aspartate; NS, not significant.

3.2. Exploratory hypotheses

GABA levels had opposite relationships to the actigraphy PLM index in the thalamus and cerebellum. The PLM index over the last two nights of the study showed a strong positive correlation with GABA levels in the thalamus in the RLS group [$r(15) = 0.73$, $P = 0.002$] (Fig. 3). On the other hand, a negative correlation between the PLM index and GABA was observed in the cerebellum on the last night of study in the RLS group [$r(15) = -0.52$, $P = 0.05$] (Fig. 4). If the subject with the very high PLM index is considered an outlier and removed from the thalamic GABA dataset, the correlation decreases to $r(14) = 0.35$ (not significant). There was no correlation between the actigraphy-derived PLM index and ACC GABA.

Polysomnographic PLM indices were not correlated with GABA levels in any of the voxels of interest. However, the PLM arousal index was negatively correlated with GABA levels in the cerebellum [$r(14) = -0.54$, $P = 0.03$]. There were no significant correlations of diary or PSQI-derived self-report sleep measures with GABA or glutamate levels in any voxel.

RLS symptom severity for the final two nights prior to MRS scans mirrored the actigraphy PLM data. The modified IRLS severity scale had a moderately positive correlation with thalamic GABA [$r(14) = 0.49$, $P = 0.055$]. Again similarly to the PLM data, RLS symptom severity showed a strong negative correlation with GABA levels in the cerebellum [$r(14) = -0.55$, $P = 0.028$]. There were no significant correlations between RLS severity while in the MRI and GABA or glutamate levels in any of the three voxels.

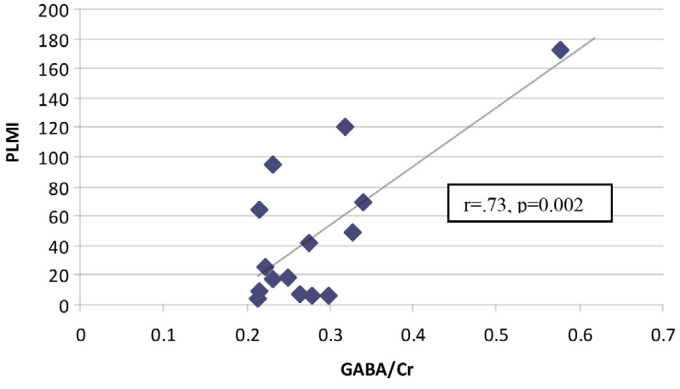

Fig. 3. Correlation of thalamic gamma-aminobutyric acid (GABA) levels with actigraphy periodic limb movement index (PLMI). Cr, creatine normalized; PLMI, number of periodic limb movements per hour in bed.

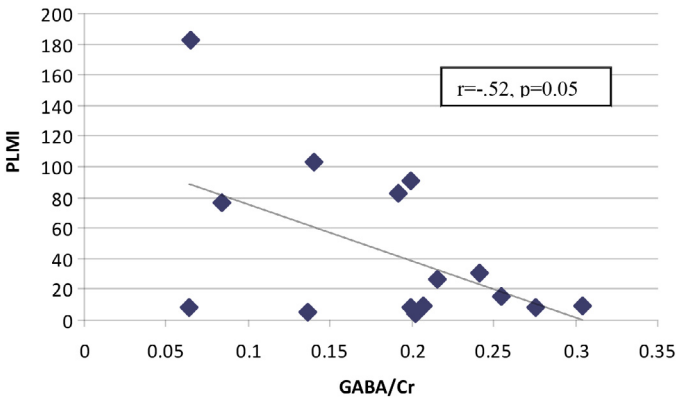

Fig. 4. Correlation of cerebellar GABA levels with actigraphy periodic limb movement index (PLMI). GABA, gamma-aminobutyric acid; CR, creatine normalized; PLMI, number of periodic limb movements per hour in bed.

4. Discussion

No group differences were found in GABA or glutamate levels between patients with RLS and matched healthy controls in the cerebellum, thalamus or ACC using ^{1}H-MRS at 4 T. However, in exploratory analyses we did demonstrate relationships between GABA levels and both RLS severity and actigraphy-derived PLM indices in patients with RLS, with positive correlations observed in the thalamus and negative correlations in the cerebellum. In addition, NAA levels were elevated in the ACC in RLS patients compared with controls.

This is the first study to examine ^{1}H-spectroscopy-derived levels of GABA in RLS. We hypothesized that GABA levels would be lower in RLS in the thalamus and ACC because these areas participate in ascending CNS pain modulatory systems [6]. Similarly, we expected lower GABA levels in the cerebellum as fMRI data suggest that this structure is active during RLS-related leg dysesthesias and movements [2,3]. There are several possible explanations for the lack of differences in GABA levels between our RLS and control groups. Our sample size was small with a maximum of 15 subjects (frequently only 13 due to data acquisition issues) in each group. However, it did not appear that increasing the sample size would influence our results, given the small between-group differences in GABA levels. It is also possible that the two-day wash-out from RLS-related medications was inadequate and that this may have influenced GABA levels in unpredictable and inconsistent ways across patients. Finally, it is possible that differences between groups may have been apparent if acquisition had occurred later in the day when those in the RLS group were more symptomatic. Supporting this possibility are the strong correlations of RLS symptom severity during the nights immediately prior to MRS scans with cerebellar and thalamic GABA levels. Consistent with this possible explanation, mean maximum RLS discomfort score in the RLS group during scanning was only 4.2 (±2.6) on a scale of 1–10, suggesting that RLS subjects were only mildly affected at the time of GABA data acquisition.

We did observe robust correlations of GABA levels with both actigraphy-derived nocturnal periodic leg movements and RLS severity in the days before the MRI. For both measures, correlations were positive in the thalamus and negative in the cerebellum. However, given that these statistical tests were not corrected for multiplicity, these results must be viewed with caution. Similarly, the association of thalamic GABA with actigraphic PLMI was substantially reduced when one subject with a very high PLMI was eliminated. The association of activity in these areas with RLS measures corroborates two previous investigations using fMRI which found cerebellar and thalamic activity associated with sensory discomfort and/or periodic limb movements [2,3]. That the correlations of GABA levels with actigraphy-related PLMS and RLS severity were in opposite directions in the thalamus and cerebellum deserves comment. Connections between the cerebellum and intralaminar nuclei of the thalamus are well established and are involved in neural traffic to the striatum [18]. The striatum has been implicated in RLS physiology (both awake and asleep) using PET, single-photon emission computed tomography, and fMRI [3,4,19]. From this perspective, overactivity of the cerebellum (associated with reduced GABA activity) could contribute to RLS symptoms through its influence on the striatum via thalamic structures. In a related study, a recent PET investigation of Tourette syndrome (which shares compulsive movements and premonitory sensory experiences with RLS) [1] also found GABA binding abnormalities in opposite directions in cerebellum and thalamus [20]. The authors of that study similarly hypothesized that tics may be generated by excessive cerebellar activity transmitted through the thalamus to the striatum.

A recent MRS study at 1.5 T found elevated thalamic glutamate levels in patients with RLS compared with controls. Glutamate levels correlated with multiple indices of sleep quality and quantity [9]. We did not find differences in glutamate levels between RLS and controls in the thalamus (or cerebellum or ACC) in the current study. Similarly, examination of the MRS Glx signal (which is composed of glutamate and glutamine), as performed by Allen et al., was no different between RLS and control groups (data not shown). There are several potential explanations for the discrepant findings of these studies. We used a GABA-optimized, 68 ms MEGA-PRESS sequence with a 4 T magnet to acquire left thalamic glutamate data in the afternoon. On the other hand, Allen used a STEAM sequence at 1.5 T, in a smaller region of the right thalamus from scans acquired in the morning. Any of these features could have led to differences in our data from that of Allen et al. In addition, although our control subjects were carefully screened to be free of RLS symptoms, we (unlike Allen et al.) did not exclude control subjects who had PLMS by polysomnography or actigraphy, which may have confounded the homogeneity of that sample.

Based on a previous study demonstrating reduced levels of NAA in the medial thalamus in RLS [10], we assessed levels of this metabolite in the thalamus, ACC and cerebellum. In distinction to the previous data we found no differences in NAA levels between RLS and controls in the thalamus, but did find a robust increase in NAA in the ACC of RLS patients compared with controls. Potential explanations for our negative finding in the thalamus are our small sample size and that we examined different regions of the thalamus than did the previous study. NAA measurements are often used as a proxy of neuronal integrity and function [21]. For this reason, our finding of higher NAA levels in the ACC in RLS patients suggests that this structure may be overactive in this disorder, which confirms previous data using fMRI [7]. As ACC activity correlates with the affective component of pain severity [22,23] and this structure may represent a hub for goal-directed responses to pain [6], our data further support this pain-like quality of RLS [1].

This study has a number of limitations. The study sample was small and thus the negative GABA and glutamate findings in the studied voxels may have been limited by statistical power. The secondary findings were exploratory and statistical tests were not corrected for multiplicity. The RLS subjects were only washed out of medications for two days, which may have led to spurious GABA or glutamate data. A habituation polysomnography night was not performed and thus a first-night effect for this data may be operative. This may then explain the relatively poor sleep quality of our control subjects in the sleep laboratory and thus the lack of statistical differences between RLS and control groups on many sleep measures.

Our negative findings regarding GABA and glutamate levels in the thalamus, ACC and cerebellum should be considered preliminary, as the small sample sizes may have limited our ability to observe true differences between groups. Similarly, our exploratory analyses demonstrating that both symptom severity and periodic limb movements in RLS are related to elevations in thalamic GABA and reductions in cerebellar GABA require confirmation. If confirmed, known cerebellar–thalamic interactions may modulate these RLS symptoms and motor findings and provide insights in the pathophysiology of RLS.

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Conflicts of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.05.019.

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